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Endothelin-A receptor antagonist BQ123 potentiates acetaminophen induced hypothermia and reduces infarction following focal cerebral ischemia in rats

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ABSTRACT

Endothelin antagonists are being investigated to prevent neuronal loss after cerebral ischemia. Acetaminophen has been tried in stroke patients to produce hypothermia so that injury following cerebral ischemia can be reduced. The aim of this study was to assess the effect of BQ123, an endothelin-A receptor antagonist, alone and in combination with acetaminophen on neurological outcome, oxidative stress and infarct volume in rats subjected to focal ischemia by occlusion of the middle cerebral artery. In normal rats, acetaminophen decreased, while BQ123 did not produce any change in body temperature, but rats treated with BQ123 and acetaminophen produced a significantly greater (41%) hypothermic response compared to acetaminophen group. In rats subjected to middle cerebral artery occlusion, neurologic deficit was observed; acetaminophen alone did not improve, but BQ123 alone and in combination with acetaminophen produced a significant improvement in neurological deficit. The level of malondialdehyde (MDA) increased and reduced glutathione (GSH) decreased in the brain following ischemia; acetaminophen did not but BQ123 alone and in combination with acetaminophen decreased MDA and increased GSH levels in ischemic rats. Cerebral ischemia produced significant infarction, the infarct volume decreased in response to BQ123 and its combination with acetaminophen. The infarct volume, MDA level and neurological deficit in ischemic rats significantly improved in rats treated with both BQ123 and acetaminophen compared to BQ123 alone. The results demonstrate that a combination of acetaminophen and BQ123 is more effective in reducing the neuronal damage following cerebral ischemia, and this combination may be worth investigating in stroke patients.

1. Introduction

Cerebral ischemia is one of the leading causes of morbidity and mortality worldwide. Hyperthermia has been associated with relatively large infarct volumes, high fatality and poor outcome, even after adjustment for initial stroke severity (Castillo et al., 1998). The period in which hyperthermia is associated with poor outcome is probably limited to the first 12 to 24 h from stroke onset (Jorgensen et al., 1996). The harmful effects of an early rise in body temperature have been attributed to increased cerebral metabolic demands, changes in the blood–brain barrier permeability, acidosis, and an increased release of excitatory amino acids (Dippel et al., 2003). In animal models of cerebral ischemia, mild intra-ischemic hyperthermia increased infarct volume, whereas mild hypothermia reduced infarct size (Schwab et al., 1998). Antipyretic agents such as acetaminophen have been used in clinical trials to decrease the body temperature of stroke patients (Dippel et al., 2003). However, the clinical benefit of using a hypothermic agent like acetaminophen was not observed, which could be due to the fact that it may not be causing sufficient decrease in body temperature.

It has been shown that endothelin is produced in the preoptic area of the anterior hypothalamus which is a thermoregulatory center (Fabricio et al., 2005). It has been shown that high levels of endothelin converting enzyme-1, endothelin-1 mRNA, and endothelin-1 are present in this area of the brain (Kurokawa et al., 2000; Sluck et al., 1999). Endothelin-1 injection in the preoptic area of the brain induces fever and appears to be important in thermoregulation (Fabricio et al., 2005, 2006). In addition, the role of central endothelin in neuronal injury due to ischemia has been reported (Willette et al., 1993). Endothelin-1 has a potent contractile effect on cerebral arteries and arterioles (Lee et al., 1990; Viossat et al., 1993), causing significant reduction in cerebral blood flow leading to infarction and neurological deficits (Robinson et al., 1990). It has been shown that endothelin plays a significant role in the pathophysiology of closed head injury and ischemic stroke, and that selectively blocking endothelin-A receptors can significantly improve neurological outcome (Barone et al., 2000) and could be effective in cerebral ischemia (Legos et al., 2008).

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Since endothelin is involved in the regulation of body temperature and in the pathophysiology of cerebral ischemia, endothelin antagonists may play a useful role in the treatment of cerebral ischemia through multiple mechanisms. It has been demonstrated that endothelin antagonists have antioxidant properties in cerebral ischemia (Briyal et al., 2007; Gupta et al., 2005; Kiris et al., 2009; Ozdemir et al., 2006). Accumulating evidence indicates a major role of free radicals and oxidative stress in the pathogenesis and pathophysiology of stroke. Oxidative stress exerts deleterious effects which include increased lipid peroxidation and decreased endogenous antioxidant capabilities. Due to the reactions involving oxygen free radicals and lipid component of cells, more stable lipid peroxidation compounds like malondialdehyde (MDA) are formed. MDA is an end product of lipid peroxidation, a measure of free radical generation. Reduced glutathione (GSH) is an essential tripeptide, an antioxidant found in all animal cells. It reacts with free radicals and can protect cells from singlet oxygen, hydroxyl radical and superoxide radical damage (Chan, 1996; Schaller, 2005). Therefore, MDA and GSH were estimated as markers of oxidative stress in the present study.

Since preliminary findings indicate that BQ123 potentiates the hypothermic effect of acetaminophen in rodents (Gulati, 2008), it is possible that a combination of acetaminophen and BQ123 may be highly effective in preventing damage due to cerebral ischemia. We have conducted this study to determine the effect of BQ123 and acetaminophen alone and in combination on body temperature, infarct volume, neurological deficit and oxidative stress parameters in middle cerebral artery occluded rats.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats weighing 300 to 350 g (Harlan, Indianapolis, IN) were used. Animals were allowed to acclimate for at least 4 days before being used in a room with controlled temperature (23 ± 1 ºC), humidity (50 ± 10%) and light (6:00 A.M. to 6:00 P.M.). Food and water were made available continuously. Animal care and use for experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Midwestern University. All anesthetic and surgical procedures were in compliance with the guidelines established by IACUC of Midwestern University.

2.2. Drugs and experimental protocol

Acetaminophen (PCCA, Houston, TX, USA) was suspended in Tween 80 (Fisher Scientific, Pittsburg, PA, USA) and injected at the dose of 300 mg/kg intraperitoneally (i.p.). The dose of acetaminophen was selected based on preliminary studies and published literature (Gulati, 2008; Ruggieri et al., 2008). BQ123, [Cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu)] (Tocris Cookson Inc., Ellisville, MO, USA) was dissolved in sterile saline and injected at the dose of 1 mg/kg, intravenously (i.v.). The dose of BQ123 was selected based on studies conducted earlier (Gulati et al., 1995, 1996). Ketamine (Hospira, Lake Forest, IL, USA) was injected at the dose of 100 mg/kg, i.p. and xylazine (Butler Animal Health Supply, Dublin, OH, USA) was injected at the dose of 10 mg/kg, i.p.

2.2.1. Experimental protocol for determination of body temperature

Rats were randomly divided into four groups consisting of 8 rats each. Group 1: vehicle (Tween 80); Group 2: vehicle + acetaminophen (300 mg/kg, i.p.); Group 3: vehicle + BQ123 (1 mg/kg, i.v.); and Group 4: BQ123 (1 mg/kg, i.v.) + acetaminophen (300 mg/kg, i.p.) group. Single injection of drugs was given in each group.

2.2.2. Experimental protocol for stroke study

Rats were randomly divided into five groups consisting of 6 rats each. Group 1: sham-operated; Group 2: vehicle (Tween 80) treated middle cerebral artery occluded rats; Group 3: acetaminophen (300 mg/kg, i.p.) treated middle cerebral artery occluded rats; Group 4: BQ123 (1 mg/kg, i.v.) treated middle cerebral artery occluded rats; and Group 5: BQ123 + acetaminophen treated middle cerebral artery occluded rats. A total of three injections of BQ123 (1 mg/kg, i.v.) and acetaminophen (300 mg/kg, i.p.) were administered; first at 30 min after middle cerebral artery occlusion, second at the time of reperfusion, and third 2 h after reperfusion.

2.3. Determination of body temperature

The change in temperature in response to acetaminophen (300 mg/kg, i.p.) or BQ123 (1 mg/kg, i.v.) alone and in combination was determined. The colonic temperature of each rat was recorded before and at various times after the injection for a period of 360 min using a Cole Palmer Animal Monitoring Thermometer with colonic probe (Vernon Hills, IL). The body temperature (ºC) was plotted with time, and data was expressed as mean ± S.E.M.

2.4. Middle cerebral artery occlusion to induce focal cerebral ischemia

The rat model of middle cerebral artery occlusion was used (Koizumi et al., 1986). Rats were anesthetized with ketamine (100 mg/kg, i.p) and xylazine (10 mg/kg, i.p.). Core temperature (rectal) was maintained at 37 ± 1 °C throughout the surgical procedure using heating lamp and the thermo-controlled base of the operating table. A midline incision was made and the right common carotid artery, external carotid artery and internal carotid artery were exposed. A 4.0 monofilament nylon thread (Ethicon, Johnson & Johnson) with its tip rounded by heating quickly by bringing it near a flame was used to occlude the middle cerebral artery. The filament was advanced from the external carotid artery into the lumen of the internal carotid artery until a resistance was felt which ensured the occlusion of the origin of middle cerebral artery. The nylon filament was allowed to remain in place for 2 h after which it was gently retracted so as to allow reperfusion of the ischemic region (Briyal et al., 2007; Gupta et al., 2005). In sham-operated rats, the external carotid artery was exposed and then the incision was sutured without touching the internal carotid artery. Rectal [core] temperature was recorded using a digital rectal thermometer and maintained at 37 °C throughout the surgical procedure using a Cole Palmer Animal Monitoring Thermometer with colonic probe (Vernon Hills, IL).

2.5. Neurological evaluation

Twenty-four hours after middle cerebral artery occlusion, the animals were subjected to neurological evaluation using a 6 point scale (Tatlisumak et al., 1998). Briefly, the scoring was as follows: 0 = no deficits, 1 = failure to extend left forepaw fully, 2 = circling to the left, 3 = paresis to the left, 4 = no spontaneous walking, and 5 = death.

2.6. Motor performance tests

Motor activity of rats was assessed 24 h after middle cerebral artery occlusion using grip test and foot fault test.

2.6.1. Grip test

Grip test was performed according to Moran (Moran et al., 1995). The apparatus with a string of 50 cm length, pulled taut between two vertical supports and elevated 40 cm from a flat surface was used. The rat was placed on the string at a point midway between supports and evaluated according to the following scale: 0 — fall off, 1 — hangs onto